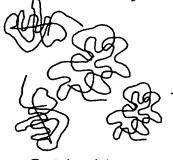
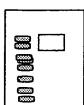
Nitrotyrosine Proteomics:

Identification of nitrotyrosine-modified proteins in complex mixtures by mass spectrometry and data-base searching

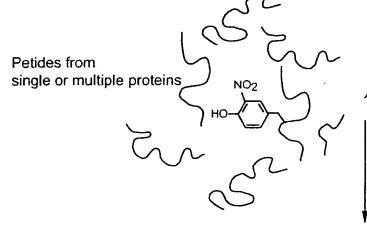


Protein mixture

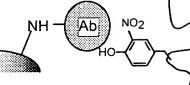
- 1. Total Protein from Sample (or immunoprecipated protein using agarose bound antinitrotyrosine AB)
- 2. Optional SDS-PAGE separation
- 3. Protease (trypsin) treatment of protein mixture or excised gel bands(s)



SDS-PAGE



Optional affinity purification with Anti-NT Abcoated Dynal Beads



(reduction)
Na₂S₂O₄

Aminotyrosine-containing peptides

NH₂

Nitrotyrosine-containing peptides and other peptides

Mass Spectrometry LC/MS

m/z

Using peak profile features unique to nitrotyrosine peptides to select peptides, i.e, MALDI triplets and/or aminotyrosine/ nitrotyrosine ($\Delta 30$ Da) ratio.

nitrotyrosine peptides

MS/MS or PSD

Mass and Sequence data for

Web-based search of protein database for protein and sitespecific identification

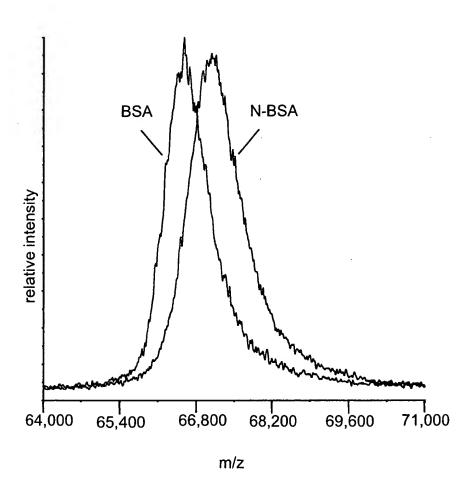


FIGURE 2

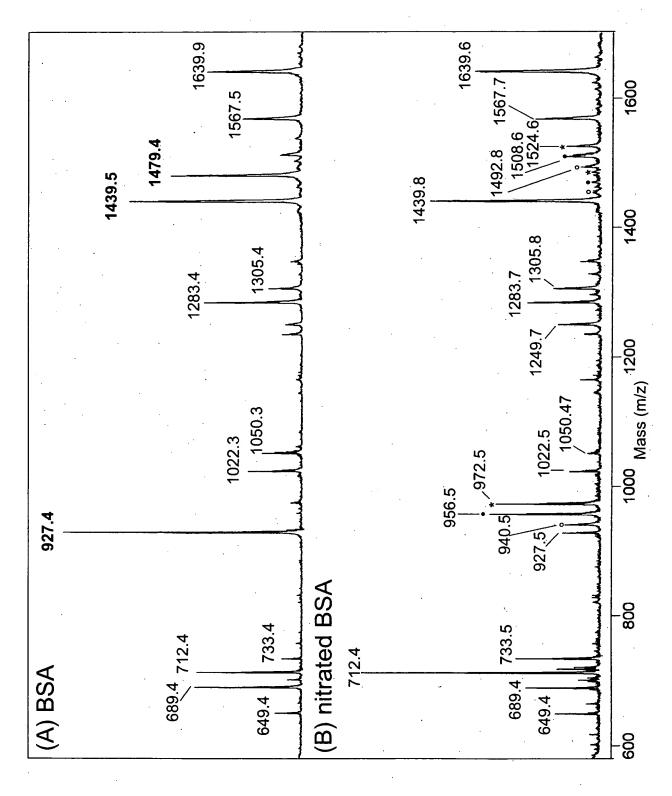
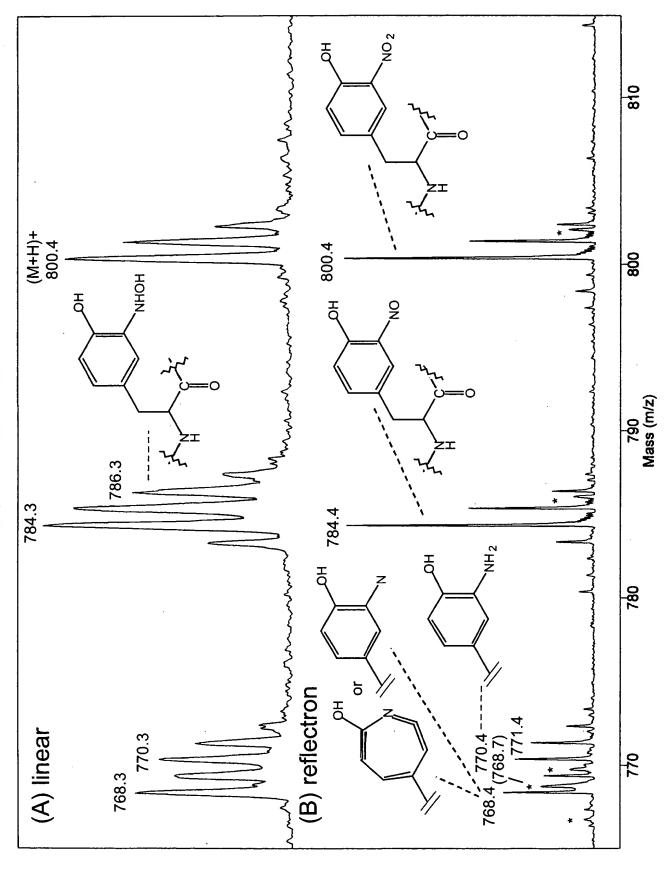


FIGURE 3



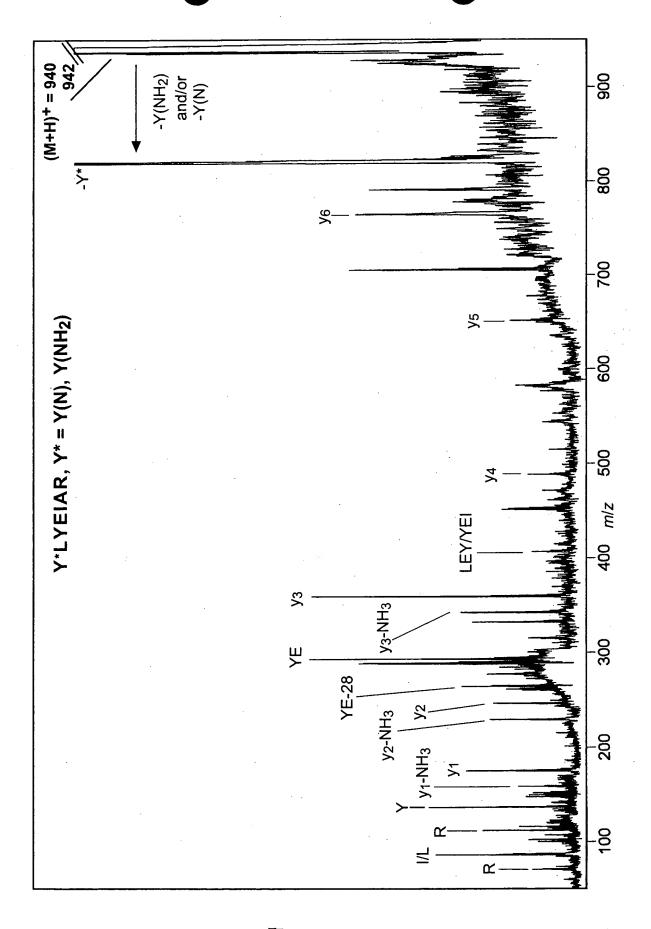
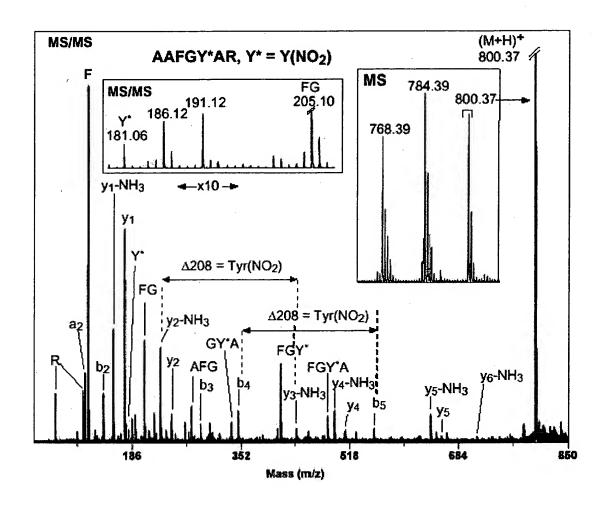
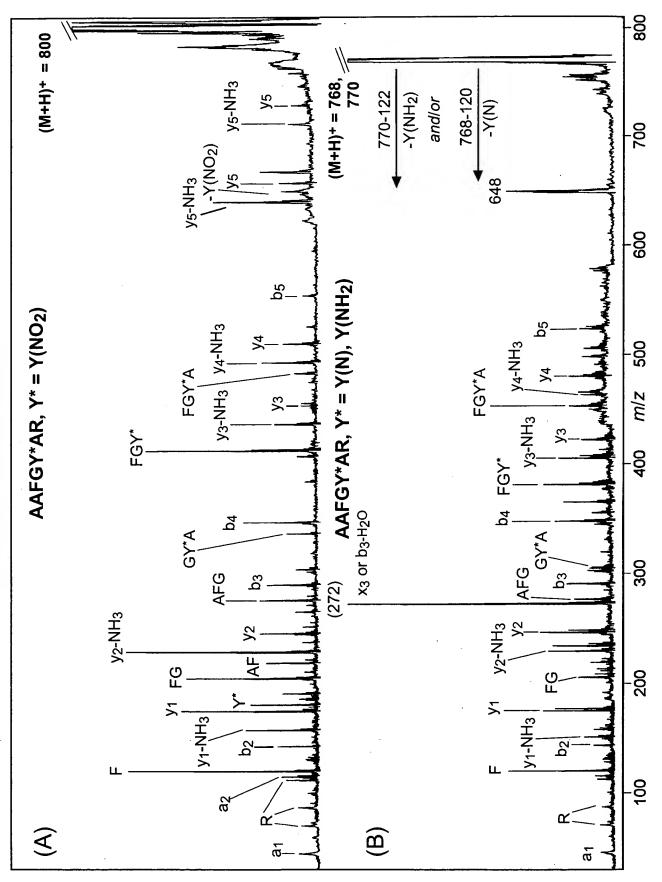


FIGURE 5





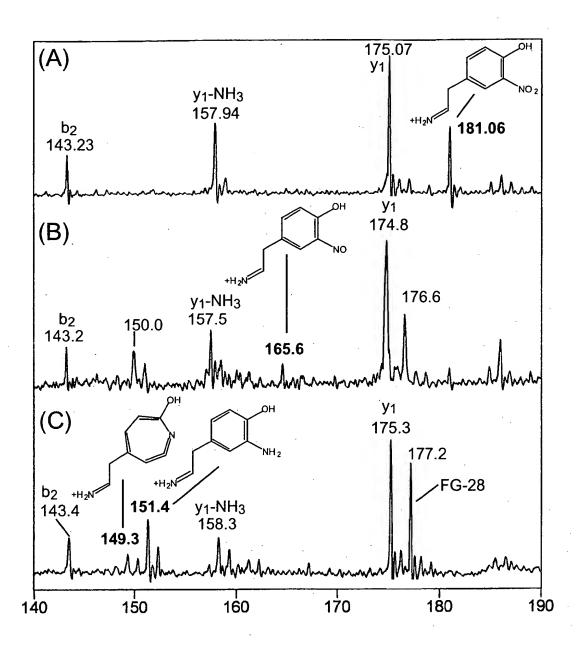


FIGURE 8

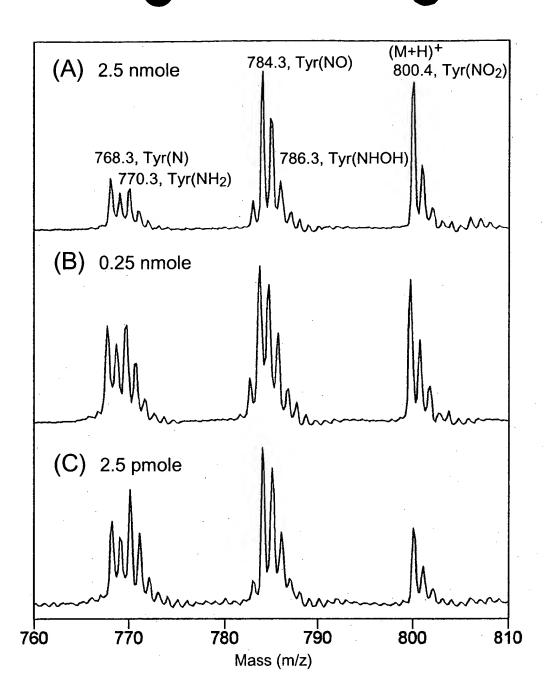


FIGURE 9

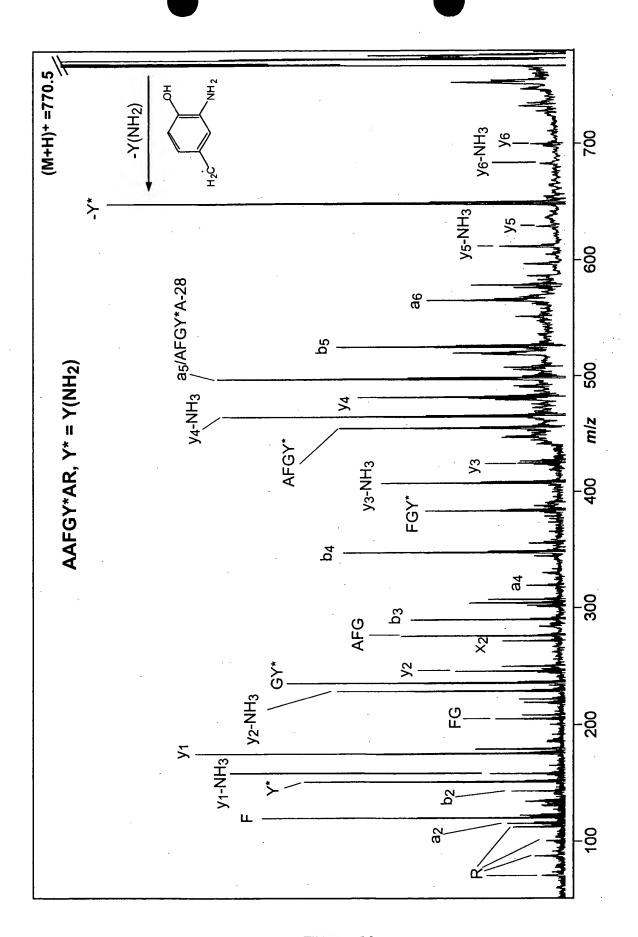


FIGURE 10